Effects of PBDE-209 exposure on primary cultures of fetal rat hippocampal neurons

Jingsi Chen, Dunjin Chen, Bo Xu, Chunfang Zhang,Yanghong Chen, Zhihua Li
Guangzhou institute of Obstetrics and Gynecology, The Third Affiliated Hospital of Guangzhou Medical College, China

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardant brominated compounds, often used as additives in resins, polystyrene and polyurethane foam, and synthetic polymer materials(Herbstman, 2007). 2',2',3',3',4',4',5',5',6',6',-decaBDE(PBDE-209) is a highly brominated diphenyl ether (PBDE) containing 10 bromine atoms, and is the most widely used flame retardant additive. In our preliminary experiments(Dunjin, 2006), we have confirmed that exposure to PBDE-209 can decrease learning and memory in mice, but the mechanism underlying these changes remains unclear. To investigate the mechanism, we exposed primary cultures of rat hippocampal neurons to PBDE-209 and assessed cell viability, the indices of oxidative stress, and apoptosis.

2. Materials and Methods

Primary cultures of fetal rat hippocampal neurons from Wistar rats (gestational age 19 days) were exposed to PBDE-209. After 7 days in culture, the cells were randomly divided into six different exposure levels of PBDE-209 as follows; the control and experimental groups A, B, C, D, E（PBDE-209 concentrations were 2.5, 5.0, 10.0, 20.0 and 40.0 ug / ml, respectively). The colorimetric MTT assay was performed to measure cell growth. Apoptotic cells were detected by flow cytometry. Measurement of superoxide dismutase (SOD)、malondialdehyde (MDA) and glutathione (GSH) levels were used to detect the effects of oxidative stress.

All results were represented as mean±standard deviation (s.d.), and statistical significance was assessed by one-way analysis of variance (ANOVA). The difference between the experimental groups and the control group were assessed by analysis of variance, in the experimental groups were assessed by least significant difference (LSD). All statistical analysis were conducted using SPSS (13.0) software. A difference of $P<0.05$ was considered statistically significant.

3. Results and Discussion

3.1 MTT assay cell viability

As shown in Table 1, the MTT colorimetric analysis assesses neuronal viability. The rate of neuronal survival decreased with increasing concentrations of PBDE-209, and the difference between the PBDE-209 treated groups and the control group was significant ($P<0.05$). The ability of concentration of PBDE-209 as low as 10ug/ml to affect viability of hippocampal neuron demonstrate that PBDE-209 can affect cell viability in vitro at low concentrations.

3.2 Effect of PBDE-209 on apoptosis

To detect whether PBDE-209 induced apoptosis, flow cytometry was used. As shown in Figure 1 and Figure 2, PBDE-209 can change the percentage of apoptotic cells ($P<0.05$). The apoptosis rate in the control group was 5.68 percent. With increasing the concentrations of PBDE-209, the rate of apoptotic cells gradually increased. Compared with the control, the percentage of apoptotic cells in Groups 30 and 40ug/ml were significantly increased.

Apoptosis is a form of cell death controlled by activation of specific genes, and plays an important role in nervous system development and maintenance. Some studies have demonstrated that PBDE congeners PBDE-47, and 99 could induce cell apoptosis (Madia, 2004). In our prior animal experiment, the pregnant mice were exposed to PBDE-209 which decreased learning in the offspring(Dunjin, 2006). We found that PBDE-209 induced apoptosis in hippocampal neurons in primary culture from fetal rats. Moreover, the degree of apoptosis was positively correlated with the concentration of PBDE-209. The result indicates apoptosis may be involved in the neurotoxic mechanism of PBDE-209.

3.3 Assessment of oxidative stress induced by PBDE-209

As shown in Table 2, PBDE-209 increased the level of MDA. The MDA content in the PBDE-209-treated groups increased with increasing PBDE concentration; the 10ug/ml、20ug/ml、40ug/ml groups were significantly higher than the control group($P< 0.05$).Compared with the control, PBDE-209 significantly decreased the SOD activity ($P<0.05$) and the GSH content ($P< 0.05$). In particular, the level of 40ug/ml group was much lower than the control.

Oxidative stress due to increased free radical generation by impaired endogenous antioxidant mechanisms is an important factor that has been implicated in neuronal damage, Alzheimer's disease, and cognitive defects seen in the elderly(Zhu. 2004; Mori 2002).Under normal circumstances, there is an equilibrium between oxidation and anti-oxidation in vivo. If this balance is disturbed, oxidative stress can lead to membrane lipid peroxidation, denaturation of proteins and enzymes, DNA damage, and cell death or apoptosis. In a study by He Weihong(He, 2008),PBDE-47 induced oxidative stress in SH-SY5Y cells. Tseng et al.reported that postnatal exposure of mice to BDE-209 could increase oxidative stress in sperm(Tseng, 2007). The present study demonstrated that when the concentration of PBDE-209 was increased, SOD activity was decreased and the content of MDA was significantly increased. At the same time, the content of GSH was also significantly decreased.These indicate that PBDE-209 could induce oxidative stress in hippocampal neurons and may be one of neurotoxic mechanisms of PBDE 209.

In conclusion, PBDE-209 caused apoptosis, increased oxidative stress in primary cultured rat hippocampal neurons. As the concentration of PBDE-209 increased, the severity of apoptosis and oxidative damage were increased. Additional studies are needed to determine other potential mechanisms of PBDE-209-induced neurotoxicity and understand these concentration-effect relationships in detail.

4. Acknowledgments

This work was supported by grants from the National Nature Science Foundation of China(NO.40590392).The authors thank Dr.Timothy J.Shafer,of the U.S. Environmental Protection Agency, for his useful comments on an earlier version of this manuscript.

5. References:

Chen Dun Jin, Yu Lin,Liao Qin-ping. 2006. Chinese journal of perinatal medicine 9: 412-415

Herbstman JB ,Sjödin A. 2007. Environ Health.115:1794-1800

He Weihong, He Ping, Wang Aiguo. 2008. Mutation Research 649:62–70

Madia F, Giordano G, Fattori V, Vitalone A, Branchi I, Capone F,Costa LG. 2004. Toxicol Lett . 154:11–21.

Mori N， Morii H. 2002. Neurosci Res. 70:264-273

Tseng LH, Lee CW, Pan MH, Tsai SS, Li MH, Chen JR, Lay JJ, Hsu PC. 2006.Toxicology.224:33–43.

Zhu XW , Raina A K, L ee HG. 2004. Brain Research. 1000: 32.

Table 1 The effect of PBDE-209 on hippocampal neurons viability（$\bar{X}$ ± s.d.）

Group	n	The rate of viability
2.5ug/ml Group	9	86.27±16.119
5.0ug/ml Group	9	77.46±18.450
10ug/ml Group	9	62.94±12.597
20ug/ml Group	9	53.75±16.088
40ug/ml Group	9	43.06±10.460
Control	9	100.00±3.009

Each exposure level had three replicates and the experiment repeated three times. The control group was treated with 1 ‰ DMSO medium.。

Table 2 The effect of BDE-209 on Oxidative stress（$\bar{X}$ ±S）

Group	N	SOD	MDA	GSH
2.5ug/ml Group	9	29.809±3.269	0.684±0.471	288.576±77.514*
5ug/mlGroup	9	28.424±1.953	1.060±0.465	257.929±70.959
10ug/mlGroup	9	26.912±1.439	1.421±0.531	126.349±40.649
20ug/mlGroup	9	25.533±2.259	1.997±0.499	76.350±13.850
40ug/ml Group	9	15.678±2.607	2.233±0.865	59.634±17.533
Control	9	38.056±4.210	0.544±0.236	347.228±38.162

Each exposure level had three replicates and the experiment repeated three times. The control group was treated with the 1 ‰ DMSO medium..

Figure 1: Induction of apoptosis by different concentrations of PBDE-209 in Flow chart

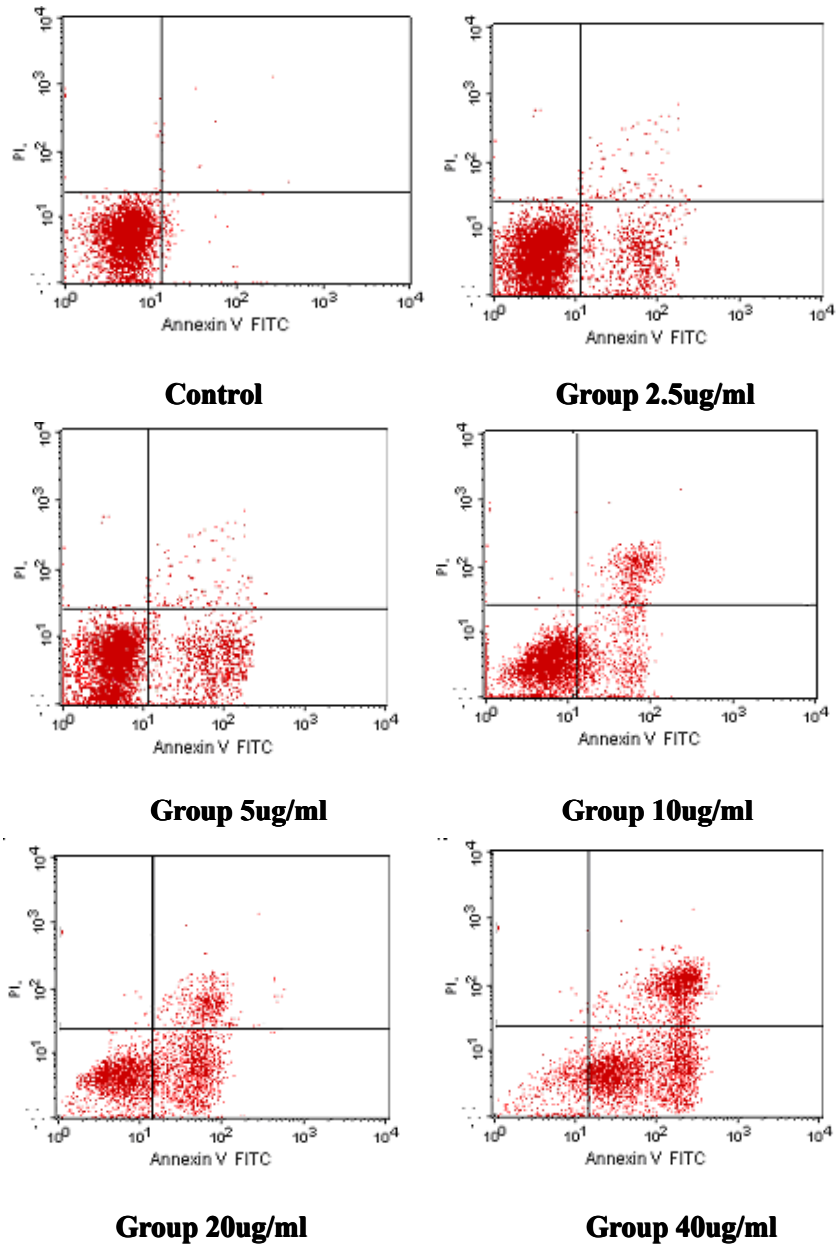

Figure 2: The effects of difference concentrations of PBDE-209 on apoptosis